

sequences of the NS RNA fused to the coding sequence of the CAT gene" with the following paragraph:

--In order to get the flanking sequences of the NS RNA fused to the coding sequence of the CAT gene, the following strategy was used. Two suitable internal restriction sites were selected, close to the start and stop codon of the CAT gene, that would allow the replacement of the sequences flanking the CAT gene in the pCM7 plasmid with the 3'- and 5'-NS RNA sequences. At the 5' end, a SfaNI site was chosen, (which generates a cut 57 nt from the ATG) and at the 3'- end a ScaI site which generates a cut 28 nt from the end of the gene (stop codon included). Next, four synthetic oligonucleotides were made using an Applied Biosystems DNA synthesizer, to generate two double-stranded DNA fragments with correct overhangs for cloning. Around the start codon these oligonucleotides formed a piece of DNA containing a XbaI overhang followed by a HgaI site and a PstI site, the 3'-(viral-sense) NS sequence immediately followed by the CAT sequence from start codon up to the SfaNI overhang (underscored). In addition a silent mutation was incorporated to generate an AccI site closer to the start codon to permit future modifications.

Xba I  
Hga I Pst I Acc I  
3' tgcgggacgtcggttttcgtccactgtttctgtattacctcttttttagtg  
SfaNI  
acccatatggtggcaactatatagggtagcgtagcatttcttg- 5' (SEQ ID NO: 21) oligo1

Xba I  
Hga I Pst I Acc I  
5'-ctagacgcctgcagcaaaagcagggtgacaaagacataatggagaaaaaatcac

SfaNI  
tgggtataccaccgttgatatatcccaatcgcatcgtaaa- 3' (SEQ ID NO: 62) oligo2

Around the stop codon the two other oligonucleotides generated a piece of DNA as follows: a blunt-ended ScaI site, the CAT sequence from this site up to and including the stop codon (underlined) followed by a BglII site and a XbaI overhang.

Sca I Bgl II  
5'-actgcatgagtgccagggcgggcgtaatagat- 3' (SEQ ID NO: 22) oligo3  
3'-tgacgctactcaccgtccccgcgcattatctagatc- 5' (SEQ ID NO: 25) oligo4

XbaI

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